Monoclonal antibodies inhibiting RNA polymerase from *Escherichia coli*

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Monoclonal hybridoma antibodies directed against RNA polymerase from *E. coli* have been obtained. Only a few have been found to inhibit the enzyme activity. Antibodies produced by two clones, PYN-1 and PYN-2, inhibit RNA polymerase at the stage of RNA chain elongation. The PYN-1 antibodies react with the β'-subunit of the enzyme. The PYN-2 antibodies react with the β-subunit and with its 130 kDa amber fragment.

RNA polymerase *RNA chain elongation* *Monoclonal antibody* *Immunoblotting*

1. INTRODUCTION

Bacterial RNA polymerases are large, complex oligomeric enzymes with the subunit composition, $d_2\beta\beta'\sigma$. The specific roles of these subunits is not yet clear. One approach to unravelling these roles is the use of antibodies as specific probes [1–3]. An obvious advantage of using monoclonal antibodies is their specificity for a single antigenic determinant [4]. Such antibodies have already been obtained against RNA polymerases from eukaryotic organisms [5–7]. Here, we describe the preparation and preliminary characterization of monoclonal antibodies inhibiting RNA polymerase from *Escherichia coli*.

2. MATERIALS AND METHODS

RNA polymerases from *E. coli* and *Pseudomonas putida* were purified by the method in [8] as described [9]. RNA polymerase activity was assayed as in [2].

BALB/c mice were immunized with 100 μg purified core RNA polymerase in a saline solution, intraperitoneally. One month later, they were boosted in the same way. Three days later the spleens were removed for fusion. The fusion with the cell line P3/Ag8.653 [10] and cloning were performed as in [11].

Screening of hybridoma lines was performed by radioimmunoassay in U-bottomed 96-well flexible microtiter plates.

For immunoblotting RNA polymerase subunits were separated on an acetate-cellulose gel in 6 M urea as in [12] and transferred to a nitrocellulose sheet by pressing. Then the nitrocellulose was treated with 5% human serum albumin in Tris-buffered saline, cut in strips, reacted for 1 h with monoclonal antibody preparations, washed, reacted with rabbit anti-mouse C_x peroxidase-conjugated antibodies, washed and developed with 3,3-diaminobenzidine in hydrogen peroxide essentially as in [13].

3. RESULTS

We have performed one fusion using spleens from 3 mice immunized with purified core RNA polymerase from *E. coli*. Growth of hybrid cells was observed in 270 out of 512 initial culture wells. 44 cell lines giving the highest radioimmunoassay counts were propagated further. Their supernatant culture fluids were screened for an ability to inhibit RNA polymerase activity. Only 3 hybridoma lines

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were found to produce inhibiting antibodies. These lines were subcloned and antibodies secreted by 2 clones, PYN-1 and PYN-2, were studied in greater detail.

A competitive radioimmunoassay showed PYN-1 and PYN-2 clones to secrete antibodies of the IgG2b type. The PYN-1 and PYN-2 antibodies neither reacted with nor inhibited RNA polymerase from Pseudomonas putida. This shows that the inhibitory action is due to specific interaction of the antibody and the enzyme, and not to contaminations. To determine the stage of transcription affected, we added antibodies into reaction mixtures either before the binding of RNA polymerase to DNA or after the beginning of RNA synthesis. Fig.1 shows that the PYN-1 antibodies obtained from ascites fluid by ammonium sulfate precipitation, inhibit RNA polymerase activity equally well in both cases. The same results were obtained for the PYN-2 antibodies. These data clearly show that the antibodies studied can inhibit RNA polymerase during RNA chain elongation but do not exclude the possibility of their affecting the preceeding stages of transcription.

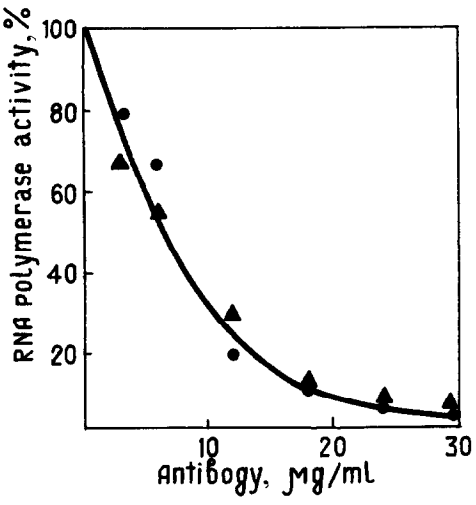

Fig.1. Inhibition of RNA polymerase activity by the PYN-1 antibodies: (●) antibodies were added to assay mixtures before the binding of RNA polymerase (20 μg/ml) to DNA; (▲) antibodies were added to assay mixtures 2 min after the beginning of RNA synthesis. To prevent reinitiation rifampicin (10 μg/ml) was also added.

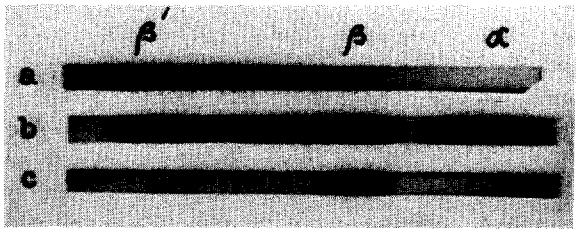

Fig.2. Interaction of antibodies with RNA polymerase subunits after their separation on acetate cellulose gel and transfer to nitrocellulose: (a) PYN-1 antibodies; (b) polyclonal antibodies; (c) PYN-2 antibodies.

To identify the subunits of RNA polymerase which contain the antigenic determinants for the monoclonal antibodies we reacted the antibodies with individual subunits separated on an acetate cellulose gel and immobilized on nitrocelluloase. Fig.2 shows that the PYN-1 antibodies react with the β′-subunit while PYN-2 antibodies react with the β-subunit.

4. DISCUSSION

We have shown rabbit polyclonal antibodies against the β- and β′-subunits (but not against the α-subunit) to inhibit RNA polymerase activity [2]. This fact suggested the involvement of the β- and β′-subunits in the catalytic function of RNA polymerase. These results confirm and extend this conclusion, which is consistent both with genetic [9,15] and with affinity-labelling data [16,17]. The localization of the relevant antigenic determinants within the polypeptide chains of the β- and β′-subunits will be useful for identifying the RNA polymerase domains involved in RNA chain elongation. So far we have shown that the PYN-2 antibodies react with the 130 kDa amber fragment of the β-subunnit in [18].

Only a minor proportion of hybridoma lines were found to produce antibodies which are inhibitory for RNA polymerase. This suggests that most antigenic determinants are located in the 'scaffolding' regions of the RNA polymerase molecule and only a few are involved in the catalytic functions of the enzyme. (Some of these functionally important sites may be located in cavities inaccessible to antibodies.)

It should be noted that all inhibitory antibodies obtained so far react with antigenic determinants

showing a rather low degree of evolutionary conservation: these antibodies do not react with RNA polymerase from *P. putida,* while almost 50% of non-inhibitory antibodies do react with the *P. putida* enzyme. This result supports our previous conclusion, based on experiments with polyclonal antibodies, that the catalytic function of most immunogenic sites of the RNA polymerase molecule is compatible with a high structural variability [2,14].

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